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BRIEF COMMUNICATIONS

Evidence for Gastrointestinal Infection of SARS-CoV-2

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Keywords: SARS-CoV-2; Gastrointestinal Infection; Oral-Fecal Transmission; ACE2.

Since the novel coronavirus (SARS-CoV-2) was identified in Wuhan, China, at the end of 2019, the virus has spread to 32 countries, infecting more than 80,000 people and causing more than 2600 deaths globally. The viral infection causes a series of respiratory illnesses, including severe respiratory syndrome, indicating that the virus most likely infects respiratory epithelial cells and spreads mainly via respiratory tract from human to human. However, viral target cells and organs have not been fully determined, impeding our understanding of the pathogenesis of the viral infection and viral transmission routes. According to a recent case report, SARS-CoV-2 RNA was detected in a stool specimen,[1] raising the question of viral gastrointestinal infection and a fecal-oral transmission route. It has been proven that SARS-CoV-2 uses angiotensin-converting enzyme (ACE) 2 as a viral receptor for entry process.[2] ACE2 messenger RNA is highly expressed and stabilized by the neutral amino acid transporter B_0AT1 (SLC6A19) in gastrointestinal system,[3,4] providing a prerequisite for SARS-CoV-2 infection. To further investigate the clinical significance of SARS-CoV-2 RNA in feces, we examined the viral RNA in feces from 71 patients with SARS-CoV-2 infection during their hospitalizations. The viral RNA and viral nucleocapsid protein were examined in gastrointestinal tissues from 1 of the patients.

Methods

From February 1 to 14, 2020, clinical specimens, including serum, nasopharyngeal, and oropharyngeal swabs; urine; stool; and tissues from 73 hospitalized patients infected with SARS-CoV-2 were obtained in accordance with China Disease Control and Prevention guidelines and tested for SARS-CoV-2 RNA by using the Chinese Center for Disease Control and Prevention–standardized quantitative polymerase chain reaction assay.[5] Clinical characteristics of the 73 patients are shown in Supplementary Table 1. The esophageal, gastric, duodenal, and rectal tissues were obtained from 1 of the patients by using endoscopy. The patient's clinical information is described in the Supplementary Case Clinical Information and Supplementary Table 2. Histologic staining (H&E) as well as viral receptor ACE2 and viral nucleocapsid staining were performed as described in the Supplementary Methods. The images of fluorescent staining were obtained by using laser scanning confocal microscopy (LSM880, Carl Zeiss MicroImaging, Oberkochen, Germany) and are shown in Figure 1. This study was approved by the Ethics Committee of The Fifth Affiliated Hospital, Sun Yat-sen University, and all patients signed informed consent forms.

Results

From February 1 to 14, 2020, among all of the 73 hospitalized patients infected with SARS-CoV-2, 39 (53.42%), including 25 male and 14 female patients, tested positive for SARS-CoV-2 RNA in stool, as shown in Supplementary Table 1. The age of patients with positive results for SARS-CoV-2 RNA in stool ranged from 10 months to 78 years old. The duration time of positive stool results ranged from 1 to 12 days. Furthermore, 17 (23.29%) patients continued to have positive results in stool after showing negative results in respiratory samples.

Gastrointestinal endoscopy was performed on a patient as described in the Supplementary Case Clinical Information. As shown in Figure 1, the mucous epithelium of esophagus, stomach, duodenum, and rectum showed no significant damage with H&E staining. Infiltrate of occasional lymphocytes was observed in esophageal squamous epithelium. In lamina propria of the stomach, duodenum, and rectum, numerous infiltrating plasma cells and lymphocytes with interstitial edema were seen.

Importantly, viral host receptor ACE2 stained positive mainly in the cytoplasm of gastrointestinal epithelial cells (Figure 1). We observed that ACE2 is rarely expressed in

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Abbreviations used in this paper: ACE, angiotensin-converting enzyme; rRT-PCR, real-time reverse transcriptase polymerase chain reaction.

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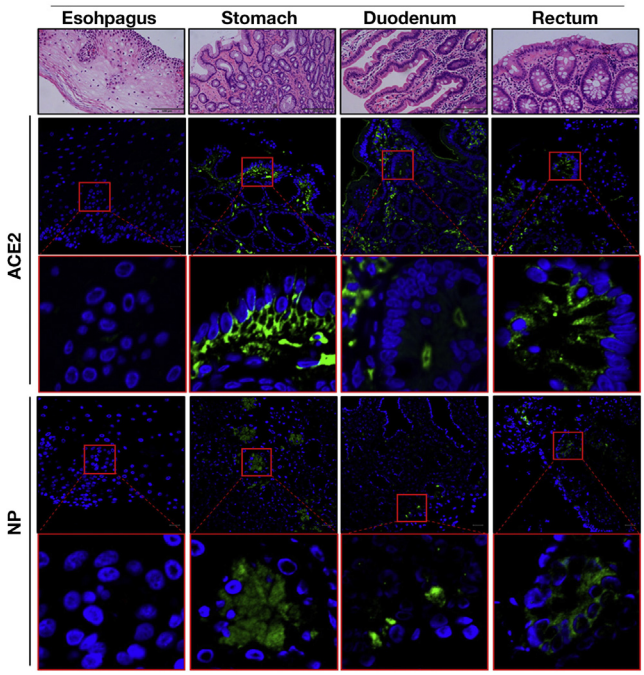

Figure 1. Images of histologic and immunofluorescent staining of gastrointestinal tissues. Shown are images of histologic and immunofluorescent staining of esophagus, stomach, duodenum, and rectum. The scale bar in the histologic image represents 100 μm. The scale bar in the immunofluorescent image represents 20 μm.

esophageal epithelium but is abundantly distributed in the cilia of the glandular epithelia. Staining of viral nucleocapsid protein was visualized in the cytoplasm of gastric, duodenal, and rectum glandular epithelial cell, but not in esophageal epithelium. The positive staining of ACE2 and SARS-CoV-2 was also observed in gastrointestinal epithelium from other patients who tested positive for SARS-CoV-2 RNA in feces (data not shown).

Discussion

In this article, we provide evidence for gastrointestinal infection of SARS-CoV-2 and its possible fecal-oral transmission route. Because viruses spread from infected to uninfected cells,[6] viral-specific target cells or organs are determinants of viral transmission routes. Receptor-mediated viral entry into a host cell is the first step of viral infection. Our immunofluorescent data showed that ACE2 protein, which has been proven to be a cell receptor for SARS-CoV-2, is abundantly expressed in the glandular cells of gastric, duodenal, and rectal epithelia, supporting the entry of SARS-CoV-2 into the host cells. ACE2 staining is rarely seen in esophageal mucosa, probably because the esophageal epithelium is mainly composed of squamous epithelial cells, which express less ACE2 than glandular epithelial cells.

Our results of SARS-CoV-2 RNA detection and intracellular staining of viral nucleocapsid protein in gastric, duodenal, and rectal epithelia demonstrate that SARS-CoV-2 infects these gastrointestinal glandular epithelial cells. Although viral RNA was also detected in esophageal mucous tissue, absence of viral nucleocapsid protein staining in esophageal mucosa indicates low viral infection in esophageal mucosa.

After viral entry, virus-specific RNA and proteins are synthesized in the cytoplasm to assemble new virions,[7] which can be released to the gastrointestinal tract. The continuous positive detection of viral RNA from feces suggests that the infectious virions are secreted from the virus-infected gastrointestinal cells. Recently, we and others have isolated infectious SARS-CoV-2 from stool (unpublished data, 2020), confirming the release of the infectious virions to the gastrointestinal tract. Therefore, fecal-oral transmission could be an additional route for viral spread. Prevention of fecal-oral transmission should be taken into consideration to control the spread of the virus.

Our results highlight the clinical significance of testing viral RNA in feces by real-time reverse transcriptase polymerase chain reaction (rRT-PCR) because infectious virions released from the gastrointestinal tract can be monitored by the test. According to the current Centers for Disease Control and Prevention guidance for the disposition of patients with SARS-CoV-2, the decision to discontinue transmission-based precautions for hospitalized patients with SARS-CoV-2 is based on negative results rRT-PCR testing for SARS-CoV-2 from at least 2 sequential respiratory tract specimens collected $\geq$24 hours apart.[8] However, in more than 20% of patients with SARS-CoV-2, we observed that the test result for viral RNA remained positive in feces, even after test results for viral RNA in the respiratory tract converted to negative, indicating that the viral gastrointestinal infection and potential fecal-oral transmission can last even after viral clearance in the respiratory tract. Therefore, we strongly recommend that rRT-PCR testing for SARS-CoV-2 from feces should be performed routinely in patients with SARS-CoV-2 and that transmission-based precautions for hospitalized patients with SARS-CoV-2 should continue if feces test results are positive by rRT-PCR testing.

Supplementary Material

Note: To access the supplementary material accompanying this article, visit the online version of *Gastroenterology* at www.gastrojournal.org, and at https://doi.org/10.1053/j.gastro.2020.02.055.

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Fei Xiao, MD, PhD (Conceptualization: Equal; Data curation: Lead; Funding acquisition: Lead; Methodology: Lead; Writing – original draft: Lead; Writing – review & editing: Lead); Meiwen Tang, MD, PhD (Conceptualization: Supporting; Data curation: Equal; Methodology: Equal; Writing – original draft: Equal; Writing – review & editing: Equal); Xiaobin Zheng, MD, PhD (Data curation: Equal; Formal analysis: Equal; Methodology: Supporting; Validation: Equal; Writing – original draft: Supporting; Writing – review & editing: Supporting); Ye Liu, MD, PhD (Conceptualization: Supporting; Data curation: Supporting; Methodology: Equal; Validation: Equal; Visualization: Equal; Writing – original draft: Supporting; Writing – review & editing: Supporting); Xiaofeng Li, MD, PhD (Conceptualization: Supporting; Methodology: Supporting; Project administration: Supporting); Hong Shan, MD, PhD (Conceptualization: Lead; Data curation: Lead; Project administration: Lead; Supervision: Lead).

Supplementary Material

Case Clinical Information

On January 17, 2020, a 78-year-old man, who, along with his wife, had come from Wuhan 6 days earlier to visit his daughter, presented to the outpatient clinic at our hospital in Zhuhai, Guangdong Province, China, with a 7-day cough and fever. He was admitted to the negative-pressure isolation room in the Department of Infectious Diseases at our hospital as a suspected case of SARS-CoV-2 infection. On admission, the physical examination showed a body temperature of 37.5°C, blood pressure of 105/56 mm Hg, pulse of 67 beats per minute, and respiratory rate of 22 breaths per minute with oxygen saturation of 97%. On physical examination, auscultation indicated rhonchi and cracks on bilateral lungs. Initial arterial blood gas analysis showed the arterial partial pressure of oxygen (Pao_2)/fraction of inspiration oxygen (Fio_2) was 176 mmHg. Nasopharyngeal and oropharyngeal swab specimens tested positive by rRT-PCR for SARS-CoV-2. Chest computed tomography presented with multiple ground-glass opacities, coinciding with previous reports,[1,2] showing evidence of pneumonia in both the left and right lungs. The patient's wife and daughter tested positive for SARS-CoV-2 RNA and were admitted to the hospital on January 18, 2020.

On hospital days 1 through 3, the patient remained febrile, with stable vital signs. The oxygen saturation remained above 95% with high-flow oxygen therapy. Empiric antimicrobials with oseltamivir and moxifloxacin was given during this period of time. On hospital day 4, the patient developed severe respiratory distress, with the Pao_2/Fio_2 decreasing to 130 mmHg and was immediately transferred to the intensive care unit, receiving an intubation and mechanical ventilation. Along with sedation, prone-position mechanical ventilation was applied for 12 hours per day, and low tidal volume was set. The Pao_2/Fio_2 increased to 350 mmHg immediately after intubation but decreased gradually again in the following several days to the lowest level of 70 at 10 days after admission. Meanwhile, the chest radiograph showed extensive bilateral consolidation; emergent veno-venous extracorporeal membrane oxygenation was applied at the same day. On day 10, coffee ground gastric contents were observed from the gastric drainage tube and fecal occult blood tested positive, indicating upper gastrointestinal bleed. Gastrointestinal endoscopy was performed to determine the exact location of bleeding. Mucosa damage in the esophagus was observed under endoscopy. Biopsy samples were taken from esophagus, gastric, duodenum, and colon for histopathologic and immunofluorescent staining. One day after treatment with octreotide, esomeprazole, etc, gastrointestinal bleeding stopped. As of February 12, 2020, the patient remained hospitalized. The vital signs were stable with mechanical ventilation, veno-venous extracorporeal membrane oxygenation, and low-dose vasopressors. There is no obvious evidence of other organ dysfunction.

Methods

Histopathologic and Immunofluorescent Staining. Esophageal, gastric, duodenal, and rectal tissues were obtained using endoscopy on day 10. Samples were embedded with paraffin and then stained with H&E. For immunofluorescent staining, 3-μm-thick sections were dewaxed in xylene, rehydrated in alcohol, and washed in distilled water 3 times before microwave repair. After washing 3 times in phosphate-buffered saline with Tween (PBST), sections were incubated with 10% goat serum in PBST for 1 hour at room temperature and then incubated overnight at 4°C with primary antibodies (anti-ACE2, Sino Biological, Beijing, China, 10108-T56, 1:500; anti-nucleoprotein, Sino Biological, 40143-T62, 1:500). The slides were incubated with secondary antibodies (Alexa Fluor 647–conjugated goat anti-rabbit IgG, bs-0296G-AF647, 1:100; Bioss, London, UK) for 1 hour at room temperature followed by washing 3 times with PBST. Nuclei were then counterstained with 4′,6-diamidino-2-phenylindole after washing 3 times with PBST. Slides were imaged by using a laser scanning confocal microscopy (LSM880, Carl Zeiss MicroImaging).

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Supplementary Table 1. Clinical Characteristics of the 73 Hospitalized Patients Infected With SARS-CoV-2

	S+	R+S+	(R+S+/S+)%	∼R+S+	(∼R+S+/R+S+)%	∼R-S+	(∼R-S+/S+)%	∼R-S-	(∼R-S-/R+S+)%
No. or % of patients	73	39	53.42%	6	15.38%	17	43.59%	16	41.03%
Female	32	14	43.75%	2	14.29%	5	35.71%	7	50.00%
Male	41	25	69.98%	4	16.00%	12	48.00%	9	36.00%
Age (years)	43 (0.83-7)	49 (0.83-78)	/	52.5 (3-78)	/	44 (0.83-69)	/	47 (19-75)	/
Tumors	7	3	42.86%	1	33.00%	1	33.00%	1	33.00%
Surgical history	17	8	47.06%	1	12.50%	4	50.00%	3	37.50%
Ulcer	0	0	/	0	/	0	/	0	/
Smoking	9	4	44%	0	0	2	50.00%	2	50.00%
Respiratory symptoms	53	30	56.60%	4	13.33%	13	43.33%	13	43.33%
Typical chest CT	66	36	54.55%	5	13.89%	16	44.44%	15	41.67%
Diarrhea	26	17	65.38%	2	11.76%	6	35.29%	9	52.94%
Gastrointestinal bleeding	10	4	40%	1	25.00%	1	25.00%	2	50.00%
Use of corticosteroid	21	12	57.14%	2	16.67%	3	25.00%	7	58.33%
Antibiotic therapy	60	35	52.05%	6	17.14%	14	40.00%	15	42.86%
Antiviral therapy	73	38	49.32%	6	15.79%	16	42.11%	16	42.11%
PPIs therapy	51	24	47.06%	4	16.67%	6	25.00%	14	58.33%
NSAID	12	6	50.00%	1	16.67%	2	33.33%	3	50.00%
ICU	4	4	100%	1	25.00%	1	25.00%	2	50.00%

CT, computerized tomography; ICU, intensive care unit; NSAID, nonsteroidal anti-inflammatory drugs; PPI, proton pump inhibitor; R, respiratory specimens; R+, SARS-CoV-2 RNA tested positive in R on hospital admission; R+S+, SARS-CoV-2 RNA tested positive in both R and S during hospitalization; ∼R+S+, SARS-CoV-2 RNA remained positive in both R and S until the date of writing the manuscript on February 14th, 2020; ∼R-S+, SARS-CoV-2 RNA converted to negative in R during hospitalization but remained positive in S until the date of writing the manuscript on February 14th, 2020; ∼R-S-, SARS-CoV-2 RNA converted to negative in both R and S during hospitalization; S, stool specimens; S+, SARS-CoV-2 RNA tested positive in stool during hospitalization; /, not applicable.

Supplementary Table 2. Timeline of Detection of Viral RNA in Different Specimens of the Patient Infected With SARS-CoV-2

Specimen	Day 1	Day 2	Day 3	Day 5	Day 7	Day 9	Day 10	Day 11	Day 13	Day 14	Day 16	Day 18	Day 20	Day 21	Day 22	Day 24	Day 26
Respiratory	NT	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive
Stool	NT	NT	Negative	Negative	Negative	Positive	Positive	Positive	Positive	Positive	Positive	NT	Positive	Positive	Positive	Positive	Positive
Serum	NT	NT	Negative	Negative	Negative	Negative	Negative	Positive	Negative	Negative	Negative	NT	NT	NT	Negative	NT	Negative
Urine	NT	NT	Negative	Negative	Negative	Negative	Negative	NT	Negative	NT	Positive	NT	NT	NT	NT	NT	NT
Esophagus	NT	NT	NT	NT	NT	NT	Positive	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
Stomach	NT	NT	NT	NT	NT	NT	Positive	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
Duodenum	NT	NT	NT	NT	NT	NT	Positive	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
Rectum	NT	NT	NT	NT	NT	NT	Positive	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT

NT, denotes not tested.